PLASMA ANGIOTENSIN II AND ALDOSTERONE EXCRETION DURING THE MENSTRUAL CYCLE

By

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ABSTRACT

The angiotensin II concentration in the plasma and the urinary aldosterone excretion were measured early in the follicular phase and late in the luteal phase in 9 women with regular menstrual cycles. The plasma angiotensin II in the follicular phase was 1.78 ng/100 ml ± 0.20 (SEM) and in the luteal phase 3.12 ng/100 ml ± 0.43 (SEM). Values for aldosterone excretion were 10.89 μg/24 h ± 1.43 (SEM) and 21.67 μg/24 h ± 2.96 (SEM) respectively. Pregnanediol increased from 0.42 mg/24 h ± 0.12 (SEM) to 2.0 mg/24 h ± 0.28 (SEM) in the same specimens of urine.

It is suggested that the observed changes in concentration and excretion of the hormones are sequentially related as follows: The sodium losing effect of progesterone stimulates renin release, giving an increased plasma angiotensin II, which in turn leads to an augmented aldosterone production.

The urinary excretion of aldosterone fluctuates significantly during the menstrual cycle (Nowaczynski et al. 1962), and Reich (1962) found that the excretion during the luteal phase was about twice that of the follicular phase. Recently it was shown that this increase was due to a similar rise in the aldosterone secretion rate (Gray et al. 1968). In view of the increased plasma renin observed during the luteal phase (Brown et al. 1964; Winer 1965; Skinner et al. 1969) it might be suggested that the progesterone of the luteal phase in some way stimulates the renin-angiotensin system, resulting in increased aldosterone secretion. The sodium excretory effect of progesterone on the kidney tubes has been demonstrated by Laidlaw et al. (1962).

The plasma angiotensin II level during the menstrual cycle has, to the best of our knowledge, so far not been investigated. If the concept presented is correct, one would expect higher angiotensin II levels in the plasma during the
luteal phase. In the present investigation the plasma concentration of angiotensin II has been measured during the follicular and luteal phase and correlated with the urinary excretion of aldosterone and pregnanediol.

**MATERIALS AND METHODS**

Nine healthy laboratory technicians, aged 18-40 years, with regular menstrual periods, on unrestricted diet, were examined in this investigation. Ovulation was calculated according to their last menstrual bleeding, and the following menstruation occurred at the expected time. A 24 h urine was collected early in the follicular and late in the luteal phase and analysed for aldosterone and pregnanediol excretion. The subjects continued with their regular work on the day of investigation. A blood sample for the determination of angiotensin II was taken between 10 and 11 a.m. on the same day on which the urine was collected, and the subjects remained sitting when the blood sample was taken.

Angiotensin II was determined as previously described (Sundsfjord 1970). Pregnanediol excretion was determined by gas phase chromatography according to Woliz (1963) except for the hydrolysis which was done with β-glucuronidase (Sigma).

The urinary aldosterone excretion was determined by a modification of a previously described gas chromatographic method (Aakvaag 1967). Tritiated aldosterone was added to a 25 ml urine portion for correction of losses encountered during the isolation procedure. Following hydrolysis at pH 1 for 24 h the urine was extracted with methylene dichloride, 40 ml three times, and the combined extract washed with 1/10 volume ice cold 0.1 N sodium hydroxide and twice with 10 ml water. The extracts were chromatographed on Whatman No. 1 paper in the systems Bush C and Bush B5 (Bush 1952). Radioactive aldosterone was detected on the chromatograms in a windowless gasflow strip counter (Actigraph III, Nuclear Chicago). The eluate after the second chromatogram was oxidized with periodic acid after evaporation of the solvent (Kitttenger 1964). The oxidizing agent was made up with 0.125 ml of 50 % periodic acid in 20 ml dioxane:water (1:1). 0.1 ml of this mixture was added to the dry residue and left at room temperature for 2½ h. 1 ml water was added and extraction carried out with 5 ml ethyl acetate. The extract was then washed three times with 0.5 ml water. The γ-lactone formed was chromatographed on thin layer of silica gel with cyclohexane:ethyl acetate (60:100) as the mobile phase. The radioactive area, corresponding to the γ-lactone, observed during scanning with a thin-layer chromatography scanner (Philips) was scraped off and eluted.

Following evaporation of the solvent, the residue was dissolved in 1 ml ethanol containing 0.2 µg corticosterone acetate, and 0.1 ml was taken out for liquid scintillation spectrometry and recovery calculation. Gas phase chromatography was carried out on a F & M model 402 chromatograph equipped with a flame ionization detector. Oxygen rather than air was used in the detector, which increases the sensitivity by a factor of 3-5 without producing an increase in noise level. The samples were chromatographed on a 90 cm column with 1% OV-17 on silanized Chromosorb W, mesh size 80/100, at 255°C with nitrogen as carrier gas at a flow rate of 60 ml per min. Under these conditions the γ-lactone had a retention time of about 7½ min, and corticosterone acetate of 12 min.

The precision of this method was calculated from the standard deviation of duplicate determinations. In 40 randomly selected pairs below 15 µg/24 h (mean 8.6) the
variance was 11.2%o, and for 40 pairs above 15 μg/24 h (mean 22.8) the variance was 10.5%o.

The accuracy was tested by adding various amounts of aldosterone between 0.38 and 1.14 μg to the urine sample. When corrections were made for losses during the isolation procedure, the average recovery of the added non-radioactive aldosterone was 98.9%o ± 7.6 (sd).

The isolation procedure with four chromatographic steps and a derivative formation was assumed to give the method adequate specificity.

Student's t-test for paired observations was used in the statistical evaluation of the results (Steel & Torrie 1960).

RESULTS

During the luteal phase a significant increase in mean aldosterone excretion and plasma angiotensin II concentration of 100 and 75%o respectively, was observed (Table 1, Fig. 1). In one subject only (E.H.) was there no increase in angiotensin II. This same subject had a surprisingly high urinary excretion of pregnanediol in the follicular phase, and no significant increase in the luteal phase (Table 1). She was also the only subject with no increase in aldosterone excretion.

DISCUSSION

The purpose of this investigation was to determine whether there was a rise in angiotensin levels which might explain the increased aldosterone excretion during the luteal phase of the menstrual cycle. Hence the aldosterone excretion and plasma angiotensin II were determined at the time of the cycle at which these values were expected to be highest and lowest respectively, i.e., late in the luteal and early in the follicular phase.

We were able to confirm the observations of Reich (1962) and Nowaczynski et al. (1962) regarding aldosterone excretion. In all but one subject there was also a rise in the angiotensin II concentration. It should be noted that the same subject had shown an abnormally high pregnanediol excretion in the follicular phase.

The increased plasma angiotensin II is in accordance with the observations of Brown et al. (1964), Winer (1965), and Skinner et al. (1969), who demonstrated an increase in plasma renin in the luteal phase. Laidlaw et al. (1962) have suggested that the increase in aldosterone might be mediated by the renin-angiotensin system.

Gray et al. (1968) found no increase in aldosterone secretion when ovulation was inhibited by oral contraceptives. The rise in aldosterone and angiotensin II reported in this paper is thus probably related to the ovarian function. It is tempting to relate the increased aldosterone production to the elevation of
Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Follicular phase</th>
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<th>Luteal phase</th>
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<tbody>
<tr>
<td></td>
<td>Subject (cycle</td>
<td>Day of cycle</td>
<td>P'diol</td>
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<tr>
<td></td>
<td>length)</td>
<td>mg/24 h</td>
<td>µg/24 h</td>
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<tr>
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<td>0.3</td>
<td>7</td>
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<tr>
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<td>14</td>
</tr>
<tr>
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<td>7</td>
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<td>15</td>
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<td>3</td>
</tr>
<tr>
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</tr>
<tr>
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<td>11</td>
</tr>
<tr>
<td>E.M.I. (28)</td>
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<td>12</td>
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<tr>
<td>E.H. (28)</td>
<td>7</td>
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<td>t (Student's t-test for paired observations)</td>
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Fig. 1.

Urinary pregnanediol and aldosterone excretion and plasma angiotensin II concentration early in the follicular and late in the luteal phase. Vertical lines represent the ± SEM.

angiotensin II. Thus if we assume that the change is caused by variations in ovarian function, then this oestrogens and/or progesterone are probably responsible for this rise.

Kinetic studies by Skinner et al. (1969) have shown that the rise in plasma renin activity during the luteal phase of the normal menstrual cycle is due to an increase in the concentration of the enzyme renin itself, without any change in its substrate, angiotensinogen. Oestrogen administration, on the other hand, leads to an increased substrate level (Douglas et al. 1969), and thus an increased plasma renin activity, as defined by the terms of the renin assay (Helmer & Judson 1967; Crane & Harris 1969). These observations suggest that the rise in oestrogens in the luteal phase is not the primary stimulus to the increased angiotensin II levels reported in this paper.

The administration of progesterone to normal male subjects increases urinary aldosterone excretion (Gornall et al. 1960). Pregnanediol excretion is a measure of the plasma progesterone concentration (Woolever 1963). A natriuretic effect of progesterone has been clearly demonstrated (Landau & Lugibihl 1958; Laidlaw et al. 1962) and progesterone has been reported to be an aldosterone antagonist (Landau & Lugibihl 1961). Laidlaw et al. (1962) have suggested that progesterone directly antagonizes the action of aldosterone on the renal tubu-
lar cell. Sodium restriction as well as drugs like thiazides (Gunnells et al. 1967) and spirolactone (Maebashi & Yoshinaga 1967), which produce an increased sodium loss, have been shown to increase plasma renin. Thus a primary increase in plasma progesterone might lead to an increased angiotensin II level as reported in this paper, through elevated sodium excretion. The augmented sodium excretion, as the consequence of elevated progesterone, will probably be fully compensated for by the sodium retaining effect of the increased aldosterone levels. Thus Gray et al. (1968) found no difference in the urinary Na/K quotient either in the follicular or in the luteal phase.

A rather remote hypothesis in this connection is that the progesterone entering the adrenal in increased amounts might possibly contribute to the substrate for aldosterone production. It might be expected that an aldosterone stimulation of this type would suppress the plasma angiotensin level, as in primary aldosteronism (Vallotton et al. 1967). The fact that increased angiotensin II was observed, makes a production of aldosterone independent of angiotensin unlikely.

According to the above considerations the following sequence is believed to occur during the luteal phase of the normal menstrual cycle. Progesterone production increases and promotes sodium loss, which in turn stimulates renin release. This leads to an increased angiotensin II formation and to stimulation of aldosterone secretion.

Whatever the mechanism, it seems advisable to take the presented results into account when women are screened for aldosteronism or when physiological studies are carried out.

REFERENCES


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